DECLARATION

I, Akinobu KOSUKEGAWA of c/o Shiga International Patent Office, 3-1, Yaesu 2 Chome, Chuo-ku, Tokyo 104-8463, Japan, understand both English and Japanese, am the translator of the English documents attached, and do hereby declare and state that the attached English documents contain an accurate translation of Ehara et al. (JP2002-355030) cited in the Office Action mailed on June 20, 2005 and that all statements made herein are true to the best of my knowledge.

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A. Kosukegawa

Akinobu Kosukegawa

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[Inventor]

[Address] No. 12-2, Shirogane 1-chome, Sakura-shi, Chiba, 285-0046, Japan

[Name] Gaku EHARA

[Inventor]

[Address] No. 32-1-1-10-502, Chiharadai 3-chome, Ichihara-shi, Chiba, 295-0158, Japan

[Name] Masaharu KIMURA

[Inventor]

[Address] No. 23-9, Ohsakidai 2-chome, Sakura-shi, Chiba, 285-0817, Japan

[Name] Hideji NISHIHASHI

[Inventor]

[Address] No. 256-3, Kami-ichiba, Mutsuzawa-machi, Chousei-gun, Chiba, 299-4403, Japan

[Name] Azusa FUJIIE

[Inventor]

[Address] No. 1740, Fuma, Yamada-machi, Katori-gun, Chiba,

289-0411, Japan

[Name] Shinichi AOYAGI

[Inventor]

[Address] No. 1-2-E-503, Oyumino-chuou-3-chome, Midori-ku, Chiba-shi, Chiba, 266-0016, Japan

[Name] Makoto HASEGAWA

[Inventor]

[Address] No. 11-12, Takahama 6-chome, Mihama-ku, Chiba-shi, Chiba, 261-0003, Japan

[Name] Masao TANAKA

[Inventor]

[Address] No. 10-1-1206, Isobe 5-chome, Mihama-ku, Chiba-shi, Chiba, 261-0012, Japan

[Name] Tomoko YOKOYAMA

[Applicant]

[ID No.] 591014710

[Name] Chiba Prefectural Goverment

[ID No.] 000002886

[Name] Dainippon Ink and Chemicals, Inc.

[Agent]

[ID No.] 100088764

[Patent Attorney]

[Name] Katutoshi TAKAHASHI

[Phone] 03-5203-7758

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PROCESS FOR PRODUCING SPORANGIA OF *BACILLUS POPILLIAE*, CONTROL AGENT, AND CONTROLLING METHOD

[CLAIM]

[Claim 1] A process for producing sporangia containing spores and parasporal bodies having a controlling effect on *Scarabaeidae* insects comprising a step of culturing microorganisms belonging to *Bacillus popilliae* in a medium, characterized in that the microorganisms are cultured in the medium containing 0.2 to 4.0% by weight of glutamic acid and 0.05 to 0.5% by weight of an absorbent.

[Claim 2] The process for producing sporangia according to claim 1 wherein said medium contains glutamic acid at 35 to 90% by weight based on total amino acids contained in the medium.

[Claim 3] The process for producing sporangia according to claim 1 wherein said medium contains pyruvic acid in the medium.

[Claim 4] A control agent for *Scarabaeidae* insects containing the sporangia of *Bacillus popilliae* containing spores and parasporal bodies obtained by the production process according to claim 1 as an active component.

[Claim 5] A controlling method for *Scarabaeidae* insects comprising a step of spraying the control agent according to claim 4 on soil where the *Scarabaeidae* insects live.

[Detailed Description of the Invention]

[0001]

[Field of the Invention] The present invention relates to a process for producing sporangia of *Bacillus popilliae* containing spores and parasporal bodies having controlling effects on *Scarabaeidae* insects by culturing microorganisms belonging to *Bacillus popilliae* in a medium, a control agent for the *Scarabaeidae* insects and a controlling method for the *Scarabaeidae* insects.

[0002]

[Prior Art] It has been known that larvae of *Scarabaeidae* insects feed on a wide range of plant roots such as those of grasses, agricultural and horticultural crops and trees, and cause considerable damage. Since these larvae live underground, in addition to it being difficult to identify the locations where these larvae are present, a high degree of control effects are unable to be obtained if pesticides or chemical pesticides are sprayed from the air. Therefore, in order to demonstrate remarkable effects, it has been necessary to spray large amounts of pesticides or chemical pesticides over a wide range to enable the chemicals to penetrate into the ground. However, since there are concerns over this method having a detrimental effect on both the natural environment and people, a more effective control method is desired.

[0003] Microorganisms belonging to *Bacillus popilliae* are known to parasitically cause milky disease in the larvae of *Scarabaeidae* insects, and eventually cause their death. Attempts have long been made to use the sporangia of these microorganisms to control *Scarabaeidae* insects on which pesticides or chemical pesticides have little effect. However, although these microorganisms grow within the larvae of *Scarabaeidae* insects, it is difficult to grow them by culturing using artificial media, and the production of sporangia of these microorganisms in media has been particularly difficult. In addition, Fukuhara has reported that infection and pathogenesis of larvae do not occur with sporangia obtained by culturing using conventional media (Fukuhara, T., ed., Insect Pathology, p. 57, 1979).

[0004] For example, Haynes et al. reported an example of obtaining a maximum of 2.06×10^7 sporangia per 1 ml of liquid culture by attempting to culture microorganisms belonging to *Bacillus popilliae* in liquid medium containing 0.5% peptone, 1.5% yeast extract, 0.3% dipotassium hydrogen phosphate, 0.1% glucose and 1% activated carbon (Journal of Invertebrate Pathology, Vol. 22, p. 377-381, 1973). However, the ratio of the content of glutamic acid to the medium and the ratio of the content of glutamic acid to total amino acids are unclear.

In addition, Haynes et al. also described in the same report that an amino acid composition is unrelated to the production of sporangia (p. 379, column 1, line 19).

[0005] In addition, Haynes et al. reported that 3.1×10^7 sporangia per 1 ml of liquid culture were obtained by culturing mature cells of *Bacillus popilliae* in the late logarithmic increase stage in liquid medium containing 0.5% peptone (tryptone), 1.5% yeast extract, 0.3% dipotassium hydrogen phosphate, 0.1% glucose and 1% activated carbon (Journal of Invertebrate Pathology, Vol. 19, p. 125-130, 1972). However, this culturing method has a long culturing time, taking roughly two weeks.

[0006] In addition, an example of having obtained 1×10^9 sporangia per 1 ml of liquid culture by culturing in liquid medium containing 1% soluble starch, 0.1% trehalose, 0.5% yeast extract, 0.3% dipotassium hydrogen phosphate and 0.1% calcium carbonate is described in US Patent No. 4,824,671. However, in this case, the resulting sporangia contain spores but no parasporal body, and the rate of infection with milky disease when the sporangia were sprayed at a rate of 2.0×10^{12} per 1 kg of soil and allowed to be orally ingested by the larvae of *Scarabaeidae* insects for 7 weeks was 47.59%, indicating weak insecticidal effects on the larvae of *Scarabaeidae* insects even

when compared with the sporangia formed within the bodies of the larvae.

[0007]

[Problems to be Solved by the Invention] An object of the present invention is to provide a process for efficiently obtaining sporangia of *Bacillus popilliae* containing spores and parasporal bodies having controlling effects on *Scarabaeidae* insects, a control agent for the *Scarabaeidae* insects obtained by the production process, and a controlling method.

[0008]

[Means for Solving the Problem] As a result of conducting extensive research to solve the aforementioned problem, the inventors of the present invention have demonstrated that effective control of the *Scarabaeidae* insects requires not only spores of *Bacillus popilliae* but sporangia containing both spores and parasporal bodies, and have found that it is necessary for the production by culture of the sporangia containing the spores and the parasporal bodies to culture in a medium to which glutamic acid and an absorbent which is thought to remove growth inhibiting substances have been added at specific concentrations, thereby leading to completion of the present invention.

[0009] That is, the present invention provides a process for

producing sporangia containing spores and parasporal bodies having controlling effects on *Scarabaeidae* insects comprising a step of culturing microorganisms belonging to *Bacillus popilliae* in a medium, characterized in that the microorganisms are cultured in the medium comprising 0.2 to 4.0% by weight of glutamic acid and 0.05 to 0.5% by weight of an absorbent.

[0010] The present invention also provides a control agent for *Scarabaeidae* insects containing the sporangia of *Bacillus popilliae* containing spores and parasporal bodies obtained by the above production process as an active component, and a controlling method for the *Scarabaeidae* insects comprising a step of spraying the control agent on soil where the *Scarabaeidae* insects live.

[0011]

[Embodiments of the Invention] The present invention will be described in detail below. In bacteriological properties of the microorganisms belonging to *Bacillus popilliae* used in the present invention in accordance with Bergey's Manual of Determinative Bacteriology, the microorganism is a gram negative bacterium with a length of 1.3 to 5.2 μm and a width of 0.5 to 0.8 μm in morphological properties, whose growth temperature is 20 to 35°C, and which has spores and parasporal bodies in sporangia.

[0012] The sporangium of the microorganism belonging to *Bacillus popilliae* is a capsule containing a spore and a small body referred to as a parasporal body (or parasporal small body) as a schematic view shown in Fig. 1. However, according to literature relating to culturing methods of *Bacillus popilliae* using conventional media, there are many examples in which sporangia and spores are used without a clear distinction, and it was often unclear as to whether the term "spore" in the literature referred to the spore alone, a sporangium that contained a spore only or a sporangium that contained a spore and a parasporal body. The inventors of the present invention have clearly determined that both the spore and the parasporal body are required in order to obtain controlling effects resulting from insecticide or growth inhibition on larvae of insects, particularly those of *Scarabaeidae* insects.

[0013] It has recently been proposed, based on the theoretical opinion of Pettersson et al. (Int. J. Syst. Bacteriol., Vol. 49, p. 531-540, 1999), that *Bacillus popilliae* including strains that have been classified thus far, should be reclassified as *Paenibacillus popilliae*, and the classification is not clearly defined at present. Thus, the microorganisms belonging to *Bacillus popilliae* in the present invention are to include microorganisms belonging to *Paenibacillus popilliae*.

[0014] The medium used in the production process of the present invention contains an absorbent for the purpose of removing substances that inhibit the growth. Examples of the absorbent include activated carbon, absorbent resin, allophosite and molecular sieve. Since hydrogen peroxide is considered to be the main substance that inhibits the growth of sporangia, the absorbent preferably has an ability to decompose or eliminate hydrogen peroxide, and specifically the activated carbon is preferably included.

[0015] Although forms of the activated carbon used for the present invention include the forms of powder, granule or sheet and the like, all of which can be used, powdered activated carbon is particularly preferable because it is superior in terms of growth efficiency and sporangium formation rate of the microorganisms.

[0016] Absorbent resins referred to in the present invention mean porous polymers that absorb microscopic substances, examples of which include crosslinked porous polymers molded into particles which function as a synthetic resin capable of efficiently absorbing microscopic substances in an aqueous solution due to the microporous structure that extends inside the particles. Specific examples can include the aromatic synthetic resin absorbents manufactured by Mitsubishi Chemical

Corporation under the trade names of Diaion HP20, Diaion HP21, Sepabeads SP825, Sepabeads SP850, Sepabeads SP70 and Sepabeads SP700, the substituted aromatic synthetic resin absorbent manufactured by Mitsubishi Chemical Corporation under the trade name of Sepabeads SP207, and the acrylic synthetic resin absorbent also manufactured by Mitsubishi Chemical Corporation under the trade name of Diaion HP2MG.

[0017] Although there is no particular restriction on the concentration of the absorbent in the medium used for the present invention provided it is within a range at which the effects of the present invention are achieved, it is preferably 0.05 to 5% by weight based on the medium. By making 0.05% by weight or more, absorption and elimination effects on substances that inhibit microorganism growth can be sufficiently exerted, and by making 5% or less, the absorption of nutrient sources required for growth of the microorganisms can be minimized, thereby resulting in the superior microbial growth promotional effects being demonstrated within the aforementioned range. The absorbent used for the present invention may be added to the medium prior to sterilization or may be added to the medium following sterilization.

[0018] The glutamic acid referred to in the present invention also includes physiologically acceptable salts thereof.

Specific examples thereof include sodium glutamate, potassium glutamate, ammonium glutamate and glutamic acid hydrochloride. The concentration of these in the medium is preferably 0.2 to 4.0% by weight as the glutamic acid, and particularly preferably 0.4 to 1.0% by weight because this is superior in microbial growth and sporangium formation rate.

[0019] Nitrogen sources required for ordinary microbial cultures are preferably added to the medium used for the present invention in addition to glutamic acid. Examples of the nitrogen sources include organic nitrogen sources used for ordinary microbial cultures such as peptone, meat extracts, fish extracts, lactoalbumin hydrolysates and yeast extracts. Examples of other nitrogen sources include inorganic nitrogen sources such as ammonia, nitric acid and salts thereof. The concentration of nitrogen sources in the medium used for the present invention is preferably 5.0% by weight or less, and more preferably 0.2 to 4.0% by weight because better microbial growth promotional effects are demonstrated.

[0020] Since the nitrogen source contains various amino acids, the addition of the nitrogen source results in glutamic acid being effectively contained in the medium. Thus, although the concentration of glutamic acid is increased by increasing the amount of the nitrogen source added, the sporangia containing

the spores and the parasporal bodies cannot be formed by this method. This is presumed to be because the concentrations of growth inhibitors and other unnecessary ingredients contained in the nitrogen source also similarly increase. Consequently, the ratio of glutamic acid to total amino acids in the medium is preferably 35 to 90% by weight.

[0021] However, the total amino acids in the present invention refer to the collection of 16 types of free amino acids composed of alanine, arginine, aspartic acid, glutamic acid, glycine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, histidine, tyrosine and valine that are known to be contained in the nitrogen sources such as peptone and yeast extract ordinarily used in media. The total amount of these 16 types of free amino acids is frequently used to roughly indicate the total amount of free amino acids contained in peptone, yeast extract and so forth.

[0022] Moreover, a carbon source normally required for microbial cultures may also be added to the medium used for the present invention. Examples of the carbon sources include sugars such as trehalose and sucrose. In addition, agricultural waste products such as molasses, starch degradation products and cheese whey can also be used. Although there is no particular restriction on the concentration at which

these carbon sources are added provided it is within a range at which the effects of the present invention are achieved, it is preferably 0.001 to 5% by weight based on the medium because this results in superior microbial growth promotional effects. However, since the presence of glucose is not preferable for forming the sporangia containing the spores and the parasporal bodies, the concentration of glucose contained in the medium is preferably 0.01% by weight or less based on the medium.

[0023] Inorganic salts such as phosphate salts such as potassium dihydrogen phosphate and dipotassium hydrogen phosphate, and sodium salts thereof may be added as necessary to the medium used for the present invention. Although there is no particular restriction on the concentration of the added inorganic salt provided it is within a range at which the effects of the present invention are achieved, it is preferably 1% by weight or less based on the medium.

[0024] Moreover, the microbial growth efficiency and the sporangium formation rate can be further enhanced by adding pyruvic acid to the medium. The pyruvic acid referred to in the present invention also includes physiologically acceptable salts thereof. Specific examples of physiologically acceptable salts of pyruvic acid include sodium pyruvate and potassium pyruvate.

[0025] The concentration of pyruvic acid is 0.01 to 0.5% by weight, and preferably 0.03 to 0.3% by weight based on the medium in terms of exhibiting the more excellent microbial growth and sporangium formation rate. Pyruvic acid added may be sterilized with medium components, or may be sterilized separately from the medium components and added at the start of the culture.

[0026] The medium used in the production process of the present invention may be a liquid medium or a solid medium. When the production process of the present invention is applied to the liquid medium, water is also to be included in the medium component. When the production process of the present invention is applied to the solid medium, base materials used preferably include polysaccharides such as agar. The concentration of the base material in the medium is 0.5 to 5% by weight, and preferably 1 to 3% by weight because this concentration results in superior microbial growth promotional effects.

[0027] A temperature suitable for growth of the microorganisms belonging to *Bacillus popilliae* used for the present invention is 25 to 32°C. In addition, the pH is 6.5 to 8.5, and more preferably 7 to 8. Examples of methods for adjusting the pH include the addition of various buffers, the addition of

routinely used acids such as hydrochloric acid and sulfuric acid, and the addition of routinely used alkalis such as sodium hydroxide, potassium hydroxide and ammonia.

[0028] Liquid culturing may be carried out by any method, examples of which include batch culture, continuous culture, semi-batch culture and feeding culture. Although culturing time varies depending on a culturing method, a culturing temperature, a culturing pH or an amount of inoculated microorganisms, it is typically 5 to 10 days in the case of the batch culture.

[0029] Following completion of the culture, the sporangia containing the spores and the parasporal bodies are recovered from the culture. Since microbial cells containing the sporangia are present on the surface of the medium in the case of the solid culture, the microbial cells are washed off by adding water or a buffer such as a phosphate buffer and Tris-HCl and suspending them, subsequently separated and recovered by a common separation method such as centrifugation and filtration. In the case of the liquid culturing, the microbial cells containing the sporangia are separated and recovered from the culture by the common separation method such as centrifugation and filtration. In the latter case, a washing procedure may be added using water or a buffer as necessary.

[0030] In the case of the culturing method using conventional media, sporangia of *Bacillus popilliae*, which contain spores and parasporal bodies that exhibit a control effect on *Scarabaeidae* insects, are hardly obtained at all, and a ratio of the number of sporangia per number of microorganisms represented by the sporangium formation rate of the equation 1 is less than 0.05%.

[Formula 1] Sporangium formation rate (%) = [(Number of sporangia)/(Number of microorganisms)] x 100

[0031] On the contrary, according to the production process of the present invention, the sporangia of *Bacillus popilliae* containing the spores and the parasporal bodies can be produced at a sporangium formation rate of 5 to 50%. In addition, 5×10^7 or more, and typically 5×10^7 to 1×10^9 sporangia containing the spores and the parasporal bodies can be produced per 1 ml of liquid culture by liquid culturing.

[0032] Examples of microorganisms belonging to *Bacillus popilliae* that exhibit growth inhibitory or insecticidal activity against *Scarabaeidae* insects include the bacterial species of *Bacillus popilliae Semadara*: FERM P-16818, *Bacillus popilliae var. popilliae Mame*: FERM P-17661, *Bacillus popilliae var. popilliae Hime*: FERM P-17660, *Bacillus popilliae var. popilliae Sakura*: FERM P-17662, *Bacillus popilliae Dutky*: ATCC

No. 14706, and *Bacillus popilliae subsp. melolonthae*.

[0033] The sporangia of *Bacillus popilliae* containing the spores and the parasporal bodies obtained by the production process of the present invention exhibit control effects such as insecticidal activity or growth inhibition on the larvae of *Scarabaeidae* insects. Consequently, the sporangia are useful as a control agent for the *Scarabaeidae* insects.

[0034] Examples of *Scarabaeidae* insects that can be controlled include *Anomala cuprea*, *Blitopertha orientalis*, *Popillia Japonica*, *Phyllopertha diversa*, *Adoretus tenuimaculatus* and *Anomala rufocuprea*.

[0035] The sporangia of *Bacillus popilliae* containing the spores and the parasporal bodies produced according to the production process of the present invention may be used as the control agent for insects, and *Scarabaeidae* insects in particular, directly in the form of a liquid in which they are suspended. Alternatively, the sporangia may be dried and sprayed in the form of powder. In addition, the sporangia may be dried followed by spraying as a suspension of water or a buffer. Moreover, the sporangia may be formulated into powders, granules, water-dispersible powders, emulsion, liquid, flowable preparation or embrocation in accordance with ordinary biocontrol agent production methods together with various

additives such as carriers, binders, dispersants, anti-freezing agents, thickeners or nutrients known publicly and used commonly for the agricultural chemicals. In addition, the sporangia containing the spores and the parasporal bodies obtained according to the production process of the present invention can also be used by mixing with other microbial preparations.

[0036] Although the content ratio of the sporangia containing the spores and the parasporal bodies contained in the aforementioned control agent varies depending on a form and a usage method of the aforementioned control agent, typically it is preferably 0.0001 to 100% by weight.

[0037] The method for applying the control agent is appropriately selected according to a preparation form, a usage method or a target crop, examples of which can include ground-level liquid spraying, ground-level solid spraying, aerial liquid spraying, aerial solid spraying, indoor application, soil mixing or soil perfusion. In addition, the control agent can also be applied by mixing with other chemicals such as insecticides, nematocides, miticides, herbicides, bactericides, plant growth regulators, fertilizers and soil improvers (such as peat, humus acid materials and polyvinyl alcohol-based materials), or can be applied alternately or

simultaneously with other chemicals without being mixed with them.

[0038] Although the applied amount of the aforementioned control agent cannot be specified unconditionally because it varies depending on the type of the *Scarabaeidae* insect, the type of an applied plant, a preparation form and the like, in the case of the ground-level spraying, for example, the applied amount of the sporangia containing the spores and the parasporal bodies of the present invention is 10^{10} to 10^{15} sporangia/a, preferably about 10^{11} to 10^{14} sporangia/a.

[0039]

[EXAMPLES] The following provides a more detailed explanation of the present invention through its examples and test examples, but the scope of the present invention is not limited thereto.

[0040] (Reference Example 1) The free amino acid contents in peptone, a yeast extract and a lactoalbumin hydrolysate used as the medium components of the medium prepared in each Example were measured by a post column method using orthophthalaldehyde (OPA).

[0041] (1) Sample preparation

Mixed amino acid standard H (manufactured by Wako Pure Chemical Industries Ltd., containing 2.5 mmol/l of each amino acid) used as the standard sample was diluted five-fold with 0.02 M

hydrochloric acid and filtered with a filter having a pore size of 0.2 μm to prepare the standard sample solution.

[0042] Measurement samples were prepared by preparing 1.0% by weight solutions of each of Polypeptone S (Nippon Pharmaceutical) or Tryptone (Difco) as peptone, and the yeast extract manufactured by Oxoid or Difco as the yeast extract, and the lactoalbumin hydrolysate (Wako Pure Chemical Industries) followed by diluting these solution two-fold with 10% by weight aqueous trichloroacetic acid solution, stirring well and centrifuging to remove any insoluble precipitations. Subsequently, the supernatant was filtered with a filter having a pore size of 0.2 μm to prepare the measurement sample solutions.

[0043] (2) Analysis

An aliquot (10 μL) of the standard sample solution or the measurement sample solution was injected into a high performance liquid chromatography to analyze the amino acids. The analysis was carried out using "LaChrom" amino acid automatic analyzer manufactured by Hitachi Ltd. based on the flow chart shown in Fig. 2. The compositions of the OPA labeling reaction solution and eluates used in the amino acid analyses are described in Tables 1 and 2, respectively.

[0044]

[Table 1]

Composition of OPA labeling reaction solution	R1	R2	R3
Boric acid		21.6g	21.6g
Sodium hydroxide	24.0g		
25% Brij-35 solution		4.0ml	4.0ml
o-Phthalaldehyde/methanol			800mg/10ml
2-Mercaptoethanol			2.0ml
5% Sodium hypochlorite solution		150.0μl	
Total volume (added distilled water up to 1.0 L)	1.0L	1.0L	1.0L

[0045] Guaranteed reagents manufactured by Wako Pure Chemical Industries were used for all reagents.

[0046]

[Table 2]

Eluate composition	A	B	C
Sodium citrate $2H_2O$	8.14g	26.67g	
Sodium chloride	7.07g	54.35g	
Citric acid H_2O	20.00g	6.10g	
Sodium hydroxide			8.0g
Ethanol	110ml		
Caprylic acid	0.1ml	0.1ml	0.1ml
Total volume (added distilled water up to 1.0 L)	1.0L	1.0L	1.0L

[0047] Reagents manufactured by Wako Pure Chemical Industries

were used for all reagents, and amino acid analytical grade reagents were used for sodium citrate $2H_2O$, citric acid H_2O and caprylic acid, while guaranteed reagents were used for all others.

[0048] The contents of L-glutamic acid and total amino acids contained in each of the measurement sample solution were calculated by converting from the peak areas obtained from the standard sample solution and each measurement sample solution, and are shown in Table 3.

[0049]

[Table 3]

	Peptone		Yeast extract		Lactoalbumin hydrolysate
	Polypeptone S	Tryptone	Oxoid	Difco	
Glutamic acid content (wt%)	0.70	1.27	7.74	7.48	2.56
Total amino acid content (wt%)	17.88	21.65	36.67	31.45	27.37

[0050] (Reference Example 2) In accordance with the culturing condition described by Haynes et al. (Journal of Invertebrate Pathology, Vol. 22, p. 377-381, 1973), namely 0.5% by weight peptone, 1.5% by weight yeast extract, 0.3% by weight dipotassium

hydrogen phosphate, 0.1% by weight glucose and 1% by weight activated carbon, the glutamic acid content and the ratio of glutamic acid to total amino acids contained in the medium prepared by using possible combinations of commercially available peptones and yeast extracts were calculated and shown in Table 4.

[0051]

[Table 4]

	Peptone used at 0.5 wt% of	Yeast extract used at 1.5 wt% of	Glutamic acid content in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)
No. 1	Polypeptone S	One from Oxoid	0.12	18.70
No. 2	Polypeptone S	One from Difco	0.12	20.62
No. 3	Tryptone	One from Oxoid	0.12	18.57
No. 4	Tryptone	One from Difco	0.12	20.44

[0052] In the case of using the commercially available peptone and yeast extract having the highest glutamic acid concentrations, namely peptone "Tryptone" from Difco and "Yeast extract" from Oxoid, the glutamic acid content in the medium was 0.12% by weight.

[0053] In addition likewise, in the case of using the commercially available peptone and yeast extract having the highest glutamic acid concentrations in the total amino acids,

namely "Polypeptone" from Nippon Pharmaceutical and "Yeast extract" from Difco, the glutamic acid content contained in the total amino acids was 20.6% by weight.

[0054] (Example 1 and Comparative Example 1) In a flask for preparation of a solid medium, 80g of distilled water was added, and L-glutamic acid (guaranteed grade manufactured by Wako Pure Chemical Industries), an absorbent, peptone ("Polypeptone S" manufactured by Nippon Pharmaceutical), trehalose dihydrate (guaranteed grade manufactured by Wako Pure Chemical Industries) and agar (guaranteed grade manufactured by Wako Pure Chemical Industries) were added thereto in amounts shown in Table 5 and mixed. With further stirring, pH was adjusted to 8.0 by adding an aqueous solution of 1 mol/l potassium hydroxide. Distilled water was further added to eventually make 100 g. Media (A-1) and (A-2) as Examples and media (B-1) to (B-4) as Comparative Examples were prepared. The activated carbon used as the absorbent was the guaranteed grade manufactured by Wako Pure Chemical Industries, and "Diaion HP20" manufactured by Mitsubishi Chemical Corporation was used as a synthetic absorbent resin (and so forth).

[0055]

[Table 5]

Medium name	Example	Comparative Example

		A-1	A-2	B-1	B-2	B-3	B-4
Medium component	L-Glutamic acid (g)	0.5	0.5	0.5	-	-	-
	Absorbent (g)	Activated carbon 0.1	Synthetic absorbent resin 2.0	-	Activated carbon 0.1	Synthetic absorbent resin 2.0	-
	Peptone (g)	0.5	0.5	0.5	0.5	0.5	0.5
	Yeast extract (g)	0.5	0.5	0.5	0.5	0.5	0.5
	Trehalose dihydrate (g)	0.1	0.1	0.1	0.1	0.1	0.1
	Agar (g)	2.0	2.0	2.0	2.0	2.0	2.0
Total volume (g) (added distilled water up to 100 g)		100					

[0056] Based on Reference Example 1, the content of glutamic acid in the medium and the ratio of glutamic acid to the total amino acids were calculated and the results are shown in Tables 6 and 7.

[0057] (Example 2 and Comparative Example 2) Culturing examples using solid media

Each medium was sterilized in an autoclave at 121°C for 20 minutes, stirred thoroughly and dispensed in 20 ml into a plastic dish with a diameter of 9 cm before the agar was solidified to make a plate medium.

[0058] Sporangia of *Bacillus popilliae Semadara* and *Bacillus popilliae var. popilliae Sakura* collected from larvae of *Scarabaeidae* insects infected with milky disease were used. The number of the sporangia was directly counted under a microscope, and a sporangium suspension was prepared using distilled water at a concentration of 1×10^7/ml. These (0.5 ml) were taken into a plastic tube, and treated with heat in a heat block at 70°C for 20 minutes. The inoculum (50 μL) was applied on the plate medium prepared above, and cultured in an incubator at 30°C for 8 days.

[0059] After completion of the culture, 2 ml of distilled water was dropped on the dish, generated colonies were thoroughly suspended, and microbial cells were collected. The numbers of sporangia and microorganisms were directly counted under the microscope, and a sporangium formation rate was calculated using the equation 1. The number of sporangia and the sporangium formation rate per dish for each microbial strain are shown in Tables 6 and 7.

[0060]

[Table 6]

Culture of *Bacillus popilliae Semadara*

Medium name	Content of glutamic acid in medium	Ratio of glutamic acid to total amino	Number of sporangia (per dish)	Sporangium formation rate (%)

	(wt%)	acids (wt%)		
A-1	0.54	70.16	2.5×10^9	42
A-2	0.54	70.16	1.0×10^9	37
B-1	0.54	70.16	0	0
B-2	0.04	15.47	5.0×10^8	13
B-3	0.04	15.47	2.0×10^8	11
B-4	0.04	15.47	0	0

[0061]

[Table 7]

Culture of *Bacillus popilliae var. popilliae Sakura*

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Number of sporangia (per dish)	Sporangium formation rate (%)
A-1	0.54	70.16	3.5×10^9	40
A-2	0.54	70.16	2.0×10^9	25
B-1	0.54	70.16	0	0
B-2	0.04	15.47	1.0×10^9	18
B-3	0.04	15.47	7.0×10^8	9
B-4	0.04	15.47	0	0

[0062] From the results in Tables 6 and 7, the number of sporangia and the sporangium formation rate were higher when cultured in the medium to which glutamic acid had been added in the presence of the absorbent for both microbial strains.

[0063] (Example 3 and Comparative Example 3) In a flask for preparation of a liquid medium, 700 g of distilled water was added, and L-glutamic acid (guaranteed grade manufactured by Wako Pure Chemical Industries) or L-alanine (guaranteed grade manufactured by Wako Pure Chemical Industries) as the added amino acid, further peptone ("Polypeptone S" manufactured by Nippon Pharmaceutical), the yeast extract (manufactured by Oxoid) and the trehalose dihydrate (guaranteed grade manufactured by Wako Pure Chemical Industries) were added thereto in amounts shown in Table 8 and mixed. With further stirring, pH was adjusted to 7.6 by adding an aqueous solution of 5 mol/l potassium hydroxide. Distilled water was further added to eventually make 850 g. This medium was transferred in a fermentor (manufactured by B. E. Marubishi) equipped with a pH electrode and sterilized in the autoclave at 121°C for 60 minutes.

[0064] Subsequently, an activated carbon dispersing solution was prepared by adding the activated carbon powder (guaranteed grade manufactured by Wako Pure Chemical Industries) in the amount shown in Table 8 in a flask and further adding distilled water to make 100 g. In addition, an antifoaming agent liquid was prepared by adding an antifoaming agent (Disfoam CA-123 manufactured by NOF Corporation) in the amount shown in Table

8 in a flask and further adding distilled water to make 50 g. The activated carbon dispersing solution and the antifoaming agent liquid were sterilized, and sterilely added in the fermentor. The medium (C-1) as Example and the media (D-1) to (D-3) as Comparative Examples were prepared.

[0065]

[Table 8]

Medium name		Example	Comparative Example		
		C-1	D-1	D-2	D-3
Medium component	Added amino acid 5 g	L-glutamic acid	L-glutamic acid	-	L-alanine
	Activated carbon (g)	3	-	3	3
	Peptone (g)	5	5	5	5
	Yeast extract (g)	5	5	5	5
	Trehalose dihydrate (g)	5	5	5	5
	Antifoaming agent (g)	1	1	1	1
Total volume (g) (added distilled water up to 1000 g)		1000			

[0066] (Comparative Example 4) In order to compare with data of Hynes et al. (Journal of Invertebrate Pathology, Vol. 22, p. 377-381, 1973), 80 g of distilled water was placed in a flask, and further peptone ("Tryptone" manufactured by Difco), the

yeast extract (manufactured by Oxoid), dipotassium hydrogen phosphate (guaranteed grade manufactured by Wako Pure Chemical Industries), glucose (guaranteed grade manufactured by Wako Pure Chemical Industries) and the activated carbon powder (guaranteed grade manufactured by Wako Pure Chemical Industries) were added in the amounts shown in Table 9 and mixed. Distilled water was further added to eventually make 100 g. This is designated as the medium (D-4). This was sterilized in the autoclave at 121°C for 20 minutes.

[0067]

[Table 9]

Medium name		Comparative Example
		D-4
Medium component	Added amino acid	-
	Activated carbon (g)	1.0
	Tryptone (g)	0.5
	Yeast extract (g)	1.5
	Glucose (g)	0.1
	Dipotassium hydrogen phosphate (g)	0.3
Total volume (g) (added distilled water up to 100 g)		100

[0068] (Example 4 and Comparative Example 5) Culturing

examples using liquid media

Sporangia previously produced in the culture using the medium (A-1) containing the activated carbon using *Bacillus popilliae Semadara*, *Bacillus popilliae var. popilliae Sakura* or *Bacillus popilliae var. popilliae Mame* as the inoculum were used. The sporangia sterilely collected were directly counted under the microscope, and a sporangium suspension was prepared at a concentration of 1×10^9 sporangia/ml using distilled water.

[0069] The sporangium suspension of each microbial strain was dispensed in 1 ml into a plastic tube, and treated with heat in the heat block at 70°C for 20 minutes. The sporangium suspension (1 ml) was inoculated into the media (C-1) and (D-1) to (D-3), and cultured for 7 days under a condition of stirring at 150 rpm, ventilation of 1 vvm, temperature at 30°C and pH at 7.6. Meanwhile, 0.01 ml of the sporangium suspension was inoculated into the medium (D-4), and cultured in the incubator at 30°C at a rotation frequency of 100 rpm for 7 days.

[0070] After the completion of the culture, the numbers of the sporangia and microorganisms per unit volume were directly counted under a microscope, and the sporangium formation rate was calculated using the equation 1. The number of sporangia per ml of the liquid culture and the sporangium formation rate are shown in Tables 10 to 12.

[0071]

[Table 10]

Culture of *Bacillus popilliae Semadara*

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Number of sporangia (per m/l)	Sporangium formation rate (%)
C-1	0.54	70.16	1.2×10^8	6.0
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0072]

[Table 11]

Culture of *Bacillus popilliae var. popilliae Sakura*

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Number of sporangia (per m/l)	Sporangium formation rate (%)
C-1	0.54	70.16	1.5×10^8	6.8
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0073]

[Table 12]

Culture of *Bacillus popilliae var. popilliae Mame*

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Number of sporangia (per m/l)	Sporangium formation rate (%)
C-1	0.54	70.16	1.6×10^8	7.2
D-1	0.54	70.16	0	0
D-2	0.04	15.47	0	0
D-3	0.04	5.76	0	0
D-4	0.12	18.60	0	0

[0074] As is evident from the results in Tables 10 to 12, the sporangia were obtained from only the media to which the absorbent and glutamic acid had been added.

[0075] (Example 5 and Comparative Example 6) Preparing examples of liquid media

In a beaker, 700 g of distilled water was placed, L-glutamic acid (guaranteed grade manufactured by Wako Pure Chemical Industries), peptone ("Polypeptone S" manufactured by Nippon Pharmaceutical), the yeast extract (manufactured by Oxoid), the lactoalbumin hydrolysate (manufactured by Wako Pure Chemical Industries) and the trehalose dihydrate (guaranteed grade manufactured by Wako Pure Chemical Industries) were added in amounts shown in Table 13, and mixed. With stirring, pH was adjusted to 7.6 by adding an aqueous solution of 5 mol/l

potassium hydroxide, and distilled water was further added to make 850 g. This medium was transferred into the fermentor (manufactured by B. E. Marubishi) equipped with a pH electrode, and sterilized in the autoclave at 121°C for 60 minutes.

[0076] Subsequently, an activated carbon dispersing solution was prepared by adding the activated carbon powder (guaranteed grade manufactured by Wako Pure Chemical Industries) in an amount shown in Table 13 into a flask and adding distilled water to make 100 g. In addition, an antifoaming agent liquid was prepared by adding an antifoaming agent (Disfoam CA-123 manufactured by NOF Corporation) in the amount shown in Table 13 in a flask and further adding distilled water to make 50 g. The activated carbon dispersing solution and the antifoaming agent liquid were sterilized, sterilely added in the fermentor, and the distilled water was further added to eventually make 1000 g. Media (E-2) to (E-6) as Examples and media (E-1) and (E-7) as Comparative Examples were prepared.

[0077]

[Table 13]

Medium name		Comparative Example	Example					Comparative Example
		E-1	E-2	E-3	E-4	E-5	E-6	E-7
Medium component	L-Glutamic acid (g)	-	0.2	0.5	0.8	1.5	3.0	5.0
	Activated carbon (g)	3	3	3	3	3	3	3
	Peptone (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	Yeast extract (g)	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	Lactoalbumin hydrolysate (g)	5	5	5	5	5	5	5
	Trehalose dihydrate (g)	5	5	5	5	5	5	5
	Antifoaming agent (g)	1	1	1	1	1	1	1
Total amount (g) (added distilled water up to 1000 g)		1000						

[0078] (Example 6 and Comparative Example 7) Culturing examples using liquid media

Sporangia previously produced in the culture using the medium (A-1) containing the activated carbon using *Bacillus popilliae Semadara* as the inoculum were used. The sporangia sterilely collected were directly counted under the microscope, and a

sporangium suspension was prepared at a concentration of 1 x 10^9 sporangia/ml using distilled water.

[0079] The sporangium suspension was dispensed in 1 ml into a plastic tube, and treated with heat in the heat block at 70°C for 20 minutes. This (1 ml) was inoculated into each medium, and cultured for 7 days under the condition of stirring at 150 rpm, ventilation of 1 vvm, temperature at 30°C and pH at 7.6. After the completion of the culture, the numbers of the sporangia and microorganisms per unit volume in the liquid culture were directly counted under a microscope, and the sporangium formation rate was calculated using the equation 1. The numbers of the sporangia and microbial cells per 1 ml of the culture and the sporangium formation rate are shown in Table 14. Relationship of the glutamic acid concentration (% by weight) in the medium with the number of microorganisms (x 10^8/ml) and the number of sporangia (x10^7/ml) is shown in Fig. 3.

[0080]

[Table 14]

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Number of microbial cells (per ml)	Number of sporangia (per ml)	Sporangium formation rate (%)
E-1	0.08	13.94	5.8×10^8	0	0
E-2	0.28	37.01	6.9×10^8	9.6×10^7	14.0

E-3	0.58	55.08	1.1×10^{9}	1.8×10^{8}	17.0
E-4	0.88	65.09	1.3×10^{9}	1.7×10^{8}	12.7
E-5	1.58	77.03	6.8×10^{8}	9.2×10^{7}	13.5
E-6	3.08	86.75	4.6×10^{8}	5.2×10^{7}	11.2
E-7	5.08	91.53	3.5×10^{8}	0	0

[0081] (Example 7 and Comparative Example 8) Preparing examples of liquid media

In a beaker, 700 g of distilled water was placed, L-glutamic acid (guaranteed grade manufactured by Wako Pure Chemical Industries), sodium pyruvate (guaranteed grade manufactured by Wako Pure Chemical Industries), peptone ("Polypeptone S" manufactured by Nippon Pharmaceutical), the yeast extract (manufactured by Oxoid), the lactoalbumin hydrolysate (manufactured by Wako Pure Chemical Industries) and the trehalose dihydrate (guaranteed grade manufactured by Wako Pure Chemical Industries) were added in amounts shown in Table 15, and mixed. With stirring, pH was adjusted to 7.6 by adding an aqueous solution of 4 mol/l sodium hydroxide, and distilled water was further added to eventually make 850 g. The prepared medium was placed into the fermentor (manufactured by B. E. Marubishi) equipped with a pH electrode, and sterilized in the autoclave at 121°C for 50 minutes.

[0082] Subsequently, an activated carbon dispersing solution was prepared by adding the activated carbon powder (guaranteed grade manufactured by Wako Pure Chemical Industries) in an amount shown in Table 15 into a flask and adding distilled water to make 100 g. In addition, an antifoaming agent liquid was prepared by adding an antifoaming agent (Disfoam CA-123 manufactured by NOF Corporation) in the amount shown in Table 15 in a flask and further adding distilled water to make 50 g. The activated carbon dispersing solution and the antifoaming agent liquid were sterilized, and then sterilely added in the fermentor. Media (F-1) and (F-2) as Examples and a medium (F-3) as a Comparative Example were prepared.

[0083]

[Table 15]

Medium name		Example		Comparative Example
		F-1	F-2	F-3
Medium component	L-Glutamic acid (g)	5.0	5.0	-
	Sodium pyruvate (g)	1.0	2.5	1.0
	Activated carbon (g)	2.5	2.5	2.5
	Peptone (g)	7.5	7.5	7.5
	Yeast extract (g)	7.5	7.5	7.5
	Lactoalbumin hydrolysate (g)	5	5	5

	Trehalose dihydrate (g)	5	5	5
	Antifoaming agent (g)	1	1	1
Total amount (g) (added distilled water up to 1000 g)		1000		

[0084] (Example 8 and Comparative Example 9) Culturing examples using liquid media

As with Example 6, *Bacillus popilliae Semadara* was used as the inoculum, and 1 ml thereof was sterilely inoculated into the media (F-1) to (F-3) to start the culture. The culture was performed under the condition of the temperature at 29°C, the ventilation of 0.5 vvm and the rotation frequency at 150 rpm, and during the culture, pH was adjusted to 7.6 using an aqueous solution of 4 mol/l sodium hydroxide and an aqueous solution of 4 mol/l sulfuric acid.

[0085] After the culture for 5 days, the numbers of sporangia and microorganisms per unit volume in the medium were directly counted under a microscope, and the sporangium formation rate was calculated. The number of microorganisms, the number of sporangia and the sporangium formation rate in the media (F-1) to (F-3) are shown in Table 16.

[0086]

[Table 16]

Medium name	Content of glutamic acid in medium (wt%)	Ratio of glutamic acid to total amino acids (wt%)	Content of pyruvic acid (wt%)	Number of microorganisms (per ml)	Number of sporangia (per ml)	Sporangium formation rate (%)
F-1	0.58	55.08	0.08	1.5×10^9	2.5×10^8	16.7
F-2	0.58	55.08	0.20	1.6×10^9	4.8×10^8	30.0
F-3	0.06	15.47	0.08	1.0×10^9	0	0

[0087] The high sporangium formation rate was achieved by adding sodium pyruvate and controlling pH, and the number of sporangia obtained was also high.

[0088] (Biological Test Example 1) A growth inhibitory effect test for larvae of *Scarabaeidae* insects by the sporangia obtained by the production process of the present invention was carried out. A suspension (I) was prepared by suspending the sporangia of *Bacillus popilliae Semadara* acquired in the medium using (A-1) of Example 2 at 2 x 10^8 sporangia/ml in distilled water. Furthermore, the suspension containing the sporangia of *Bacillus popilliae Semadara* acquired in the medium using (A-1) of Example 2 was treated with French press to separate and remove spores and parasporal bodies from the sporangia. A suspension (II) was prepared by suspending the separated spores at 2 x 10^8 spores/ml in distilled water. In addition, a suspension (III) was prepared by suspending the separated parasporal bodies

at 2 x 10^8 parasporal bodies/ml in distilled water.

[0089] Eighty plastic cups with a diameter of 6 cm in which about 20 g of leaf mold had been placed were prepared.

(i) The suspension (I) containing the sporangia was sprayed at 2 x 10^8 sporangia per cup onto 20 plastic cups.

(ii) The suspension (II) containing only the spores was sprayed at 2 x 10^8 spores per cup onto 20 plastic cups.

(iii) The suspension (III) containing only the parasporal bodies was sprayed at 2 x 10^8 parasporal bodies per cup onto 20 plastic cups.

(iv) Nothing was sprayed onto remaining 20 plastic cups to use as controls.

One second instar larva each of *Anomala cuprea* was placed in each cup and bred for 30 days in an incubator at 25°C followed by measuring the mortality rates and average body weight gain of the surviving larvae over time. The cumulative mortality rates are shown in Table 17, while the results for growth inhibitory effects are shown in Fig. 4.

[0090]

[Table 17]

	Cumulative mortality rate (%)		
Test group	Day 11	Day 23	Day 30
i)	20	40	45

ii)	0	5	10
iii)	15	20	25
Control	0	0	0

[0091] Based on the above results, the sporangium containing both the spore and the parasporal body was confirmed to demonstrate superior insecticidal and larva growth inhibitory effects.

[0092] (Biological Test Example 2) A test was conducted for the insecticidal activity on *Scarabaeidae* insects by sporangia obtained according to the production process (solid culture) of the present invention.

[0093] Sporangia of *Bacillus popilliae Semadara* acquired in the culture using the activated carbon-containing plate medium (A-1) in Example 2 were suspended at 1×10^9 sporangia/ml in distilled water to prepare a sporangium suspension. Approximately 20 g each of leaf mold was placed in 40 plastic cups with a diameter of 6 cm, and the sporangium suspension was sprayed onto 20 of those cups so that the number of sporangia was 1×10^9 sporangia/cup. The sporangium suspension was not sprayed onto the remaining 20 cups and these cups were used as the control test. One second instar larva each of *Anomala cuprea* was placed in each cup and bred for 40 days in the incubator at 25°C followed by investigating the number of insects that

died over time to determine the cumulative mortality rate (%).

[0094] The insecticidal activity on *Anomala cuprea* by the sporangia obtained by the solid culture of the present invention is shown in Table 18. The mortality rate of 100% was observed on Day 40.

[0095]

[Table 18]

	Cumulative mortality rate (%)			
Test group	Day 10	Day 20	Day 30	Day 40
Control	0	0	0	0
Addition of sporangia	40	60	90	100

[0096] (Biological Test Example 3) A test was conducted for the insecticidal activity on *Scarabaeidae* insects by sporangia obtained according to the production process (liquid culturing) of the present invention. The test groups were made in the same way as in Biological Test Example 2. However, the sprayed sporangia composed of

(i) sporangia of *Bacillus popilliae Semadara* acquired in the culture using the activated carbon-containing liquid medium (C-1) of Example 4, and

(ii) sporangia of *Bacillus popilliae var. popilliae Mame* acquired in the culture using the activated carbon-containing

liquid medium (C-1) of Example 4. One second instar larva each of *Anomala cuprea* was placed in each cup and bred for 40 days in the incubator at 25°C followed by investigating the number of insects that died over time to determine the cumulative mortality rate (%).

[0097] The insecticidal activity on *Anomala cuprea* by the sporangia obtained by the liquid culturing of the present invention is shown in Table 19. The mortality rate of 85 to 100% was observed on Day 40.

[0098]

[Table 19]

	Cumulative mortality rate (%)			
Test group	Day 10	Day 20	Day 30	Day 40
Control	0	0	0	0
i)	15	30	95	100
ii)	10	35	65	85

[0099] (Biological Test Example 4) A test was conducted for the insecticidal activity on *Scarabaeidae* insects by sporangia obtained according to the production process (liquid culturing) of the present invention. Sporangia of *Bacillus popilliae Semadara* acquired in the culture using the medium (F-2) in Example 8 were suspended at 1 x 10^9 sporangia/ml in distilled

water to prepare a sporangium suspension.

[0100] Approximately 20 g each of leaf mold was placed in 40 plastic cups with a diameter of 6 cm, and the sporangium suspension was sprayed onto 20 of those cups so that the number of sporangia was 1 x 10^9 sporangia/cup. The sporangium suspension was not sprayed onto the remaining 20 cups and these cups were used as the control test. One second instar larva each of *Anomala cuprea* was placed in each cup and bred for 40 days in the incubator at 25°C followed by investigating the number of insects that died over time to determine the cumulative mortality rate (%).

[0101] The insecticidal activity on *Anomala cuprea* by the sporangia formed out of insect bodies is shown in Table 20. The obtained sporangia exhibited the insecticidal activity, and all larvae died within 40 days.

[0102]

[Table 20]

	Cumulative mortality rate (%)			
Test group	Day 10	Day 20	Day 30	Day 40
Control	0	0	0	0
Addition of sporangia	15	30	95	100

[0103]

[Effects of the Invention] The present invention can provide the production process by which the sporangia of *Bacillus popilliae* containing spores and parasporal bodies are efficiently yielded. That is, the present invention can produce the sporangia of *Bacillus popilliae* containing the spores and the parasporal bodies at a sporangium formation rate of 5 to 50% by culturing in the liquid medium for about 5 to 10 days. In addition, the present invention can produce the sporangia of *Bacillus popilliae* containing the spores and the parasporal bodies at a rate of 5×10^7 or more sporangia per ml of the liquid culture. The present invention can provide the control agent which exhibits the insecticidal effects on insects, particularly *Scarabaeidae* insects and the growth inhibitory effects on larvae thereof, and the controlling method of the insects, particularly the *Scarabaeidae* insects using the control agent.

[Brief Description of Drawings]

Fig. 1 is a schematic view showing a sporangium of *Bacillus popilliae* containing a spore and a parasporal body;

Fig. 2 is a schematic view showing a high performance liquid chromatography system used for amino acid analysis;

Fig. 3 is a graph showing numbers of sporangia and microbial cells for glutamic acid concentrations in a medium in Example

3; and

Fig. 4 is a graph showing growth inhibitory effects on *Anomala cuprea* in Biological Test Example 1.

[Description of Signs]

1. Sporangium
2. Parasporal body
3. Spore

[ABSTRACT]

[Problems to be Solved] To provide a process for efficiently producing sporangia of *Bacillus popilliae* containing spores and parasporal bodies having controlling effects on *Scarabaeidae* insects, and a control agent and a controlling method for *Scarabaeidae* insects obtained by the production process.

[Means for Solving the Problems] A process for producing sporangia containing spores and parasporal bodies by culturing microorganisms belonging to *Bacillus popilliae* in a medium, wherein the microorganisms are cultured in the medium containing 0.2 to 4.0% by weight of glutamic acid and 0.05 to 0.5% by weight of an absorbent and the sporangia of *Bacillus popilliae* containing spores and parasporal bodies having a controlling effect on *Scarabaeidae* insects is provided. Furthermore, a control agent containing the sporangia of *Bacillus popilliae* containing spores and parasporal bodies obtained by the above process as an active component, and a controlling method of *Scarabaeidae* insects wherein the control agent is sprayed on soil where the *Scarabaeidae* insects live.

[Amendments]

[Submitted on] July 2, 2002

[Amendment 1]

[Title of Document to be Amended] Specification

[Title of Item to be Amended] 0032

[Method for Amendment] Change

[Details of amendment]

[0032] Examples of microorganisms belonging to *Bacillus popilliae* that exhibit growth inhibitory or insecticidal activity against *Scarabaeidae* insects include the bacterial species of *Bacillus popilliae Semadara*: FERM BP-8068, *Bacillus popilliae var. popilliae Mame*: FERM BP-8069, *Bacillus popilliae var. popilliae Hime*: FERM P-17660, *Bacillus popilliae var. popilliae Sakura*: FERM P-17662, *Bacillus popilliae Dutky*: ATCC No. 14706, and *Bacillus popilliae subsp. melolonthae*. *Bacillus popilliae Semadara* was deposited to Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human-Technology (at present, International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology) with the accession number of FERM P-16818 on May 21, 1998, and has been deposited under the international accession number FERM BP-8068 by transferring to the international deposit in

accordance with the Budapest Treaty on May 21, 2002. *Bacillus popilliae var. popilliae Mame* was deposited to Ministry of International Trade and Industry, Agency of Industrial Science and Technology, National Institute of Bioscience and Human-Technology (at present, International Patent Organism Depositary, National Institute of Advanced Industrial Science and Technology) with the accession number of FERM P-17661 on November 25, 1999, and has been deposited under the international accession number FERM BP-8069 by transferring to the international deposit in accordance with the Budapest Treaty on June 10, 2002.

Fig. 1

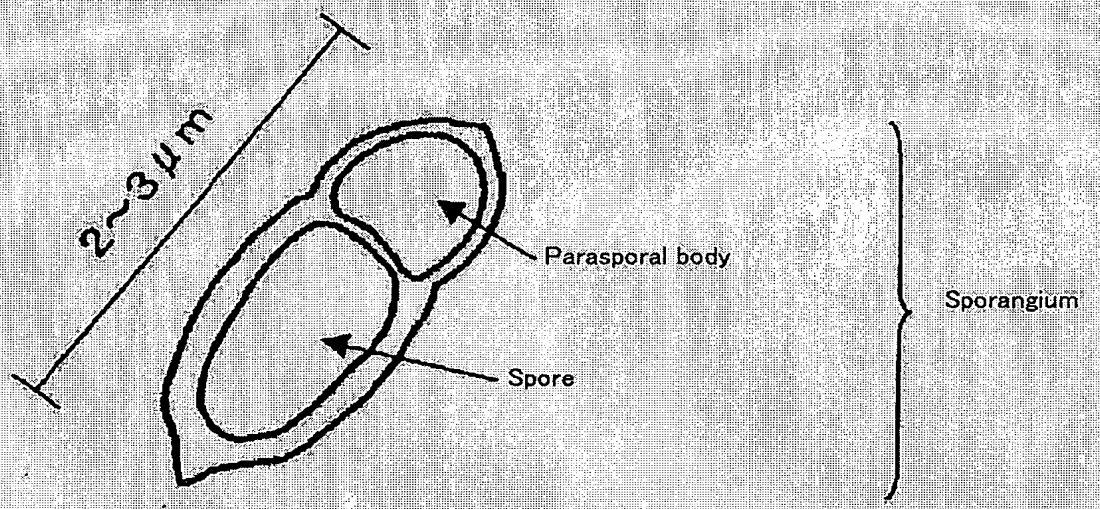

Fig. 2

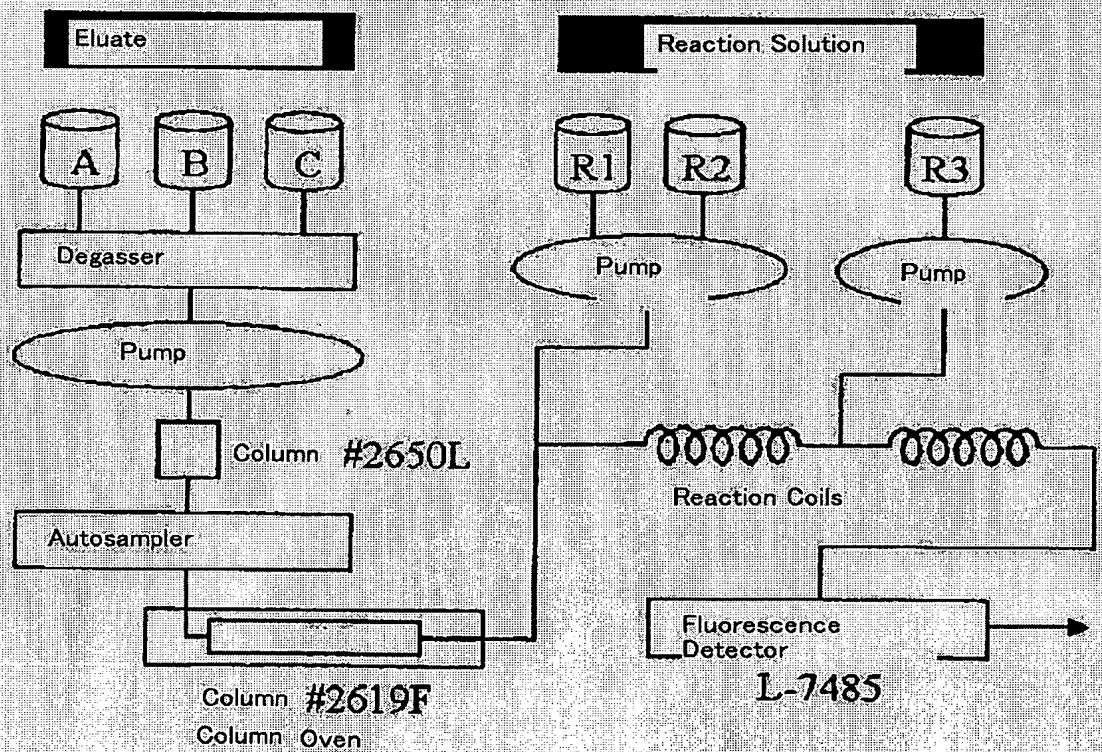

Fig. 3

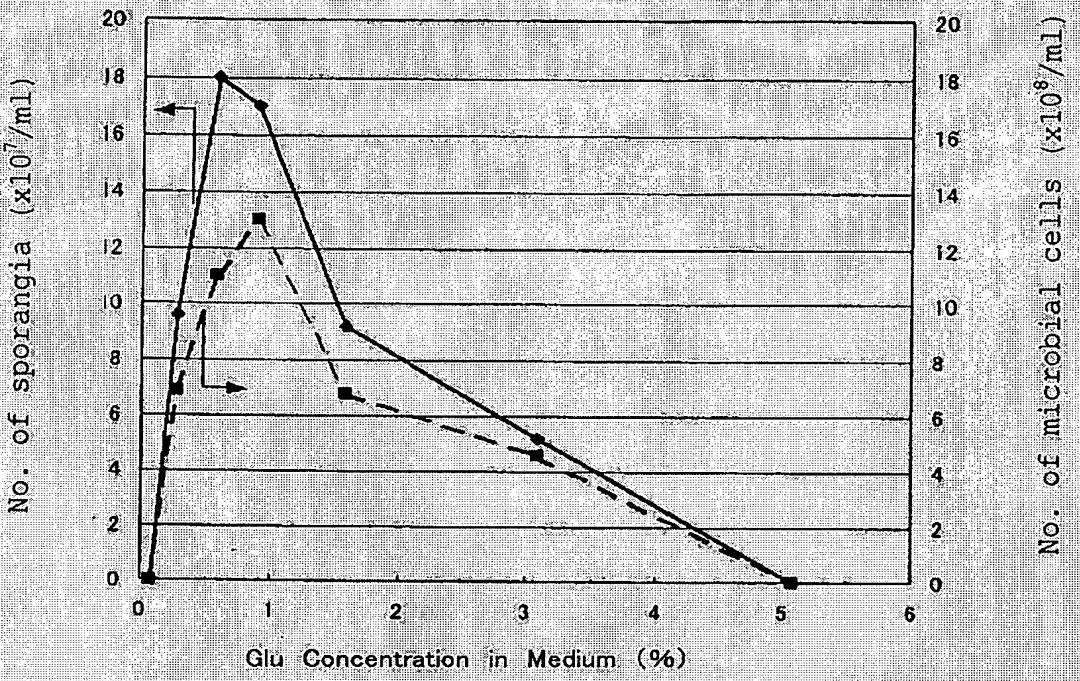

Fig. 4